THE UNIVERSITY OF WISCONSIN
MADISON 6

Institute for Enzyme Research
1702 University Avenue

Sep. 25, 1961.

Dear Maxine:

I want first of all to tell you how deeply I enjoyed working with you and that I am deeply indebted to you for all the time and attention you gave me. My stay there was so profitable to me materially and spiritually that I wish I could return there. Of course, I also hope that I would be able sometime to have you and/or members of the group here. Thanks again for so much.

I got your note and the survey of my preparation. it was all due to your expert supervision that it came out so well.

Fritz Lipmann wanted to try out Poly U and asked me about the preparation of the phosphoglose and I felt that the best I could do was to refer him to the the originator and I hope this did not cause you any inconvenience. I am sure your procedure is going to be heavily in demand.

As regards the use of pyridinium Dowex 50 for removing KOH and all $K^+$ ions from hydrolysate, this is very simple. We first clean the commercial Dowex 50 ($H^+$) by NaOH then excess of HCl cycle - till no NaCl comes off - wash thoroughly with $H_2O$ & keep it in the acidic form. With passage of time this resin releases some low mol. wt colored polymers & when I come to using it for making $Py^+H$ form we pack some mls. of wet resin in a little column & put 10 - 20 % Pyridine through it & wash it for ½ hours or so with water. Russell did it while I was there. This freshly prepared Py$^+$H resin pyridinium ($Py^+_H$) resin can be used either in drained form by adding it to the KOH hydrolysate

or by passing the KOH hydrolysate through a column of the resin & washing the column with 5% aq. Pyridine. For 1 mmole of KOH ca 2-3 ml. of resin is more than enough. excess of resin doesn't matter of course. The eluate from column now contains the nucleotide as pyridine salts and pyridine. If you wish to concentrate it by evaporation, pyridine goes off first. pH is likely to drop down to about 3. Use this aq. solution for any purpose you want.

For small scale, as I suggested to Leon, one could add $Py^+H$ resin to the aliquots of alkaline digest & spin down the resin (it packs quite nicely) and use supernate for analysis.

If there are any questions let me know.

Separately I am sending a copy of my little book. Hope you find something of interest in it.

With all the best of regards to you and Dan & love to children.

Govind.